REMARKS

Rejection of Claims on Enablement Grounds in the 02 June 2004 Office Action and Traversal Thereof

In the previous 02 June 2004 Office Action, the Examiner rejected claims 1-6 under 35 U.S.C. 112 for failing to comply with the Enablement requirement. In response to this rejection, the claims have been amended to more clearly state the invention. The Examiner also duly objected to mistakes present in the specification. Amendments have been made to the specification to correct these mistakes and to overcome the objections of the Examiner. Furthermore, Figures 4 and 6 have been corrected to overcome the Examiner's objections. The above rejections on the Enablement grounds are traversed, and consideration of the patentability of the claims 1-37, now amended, is requested, in light of the following remarks.

Arguments for Patentability

The Examiner has rejected claims 1-6 under 35 U.S.C. 112 as not describing the invention in such a way as to enable one skilled in the art to make and use the invention. To address this concern, the Applicant has amended the claims to more fully and clearly state the invention. The present invention relates to a method of immunizing a host animal against a gastrointestinal, mucosally invasive *Mycobacterium avium* subspecies *paratuberculosis* (MAP) by orally administering ***a live, attenuated strain of MAP*** to the host animal. The attenuated MAP strain would be capable of generating a prolonged Th1- and IgA-dependent immunological response in the intestinal mucosa so as to further elicit a local cell-mediated immune response having a long-term immunological memory

and capable of withstanding a challenging dose of wild, disease-causing MAP strains. This approach is distinguished over prior work by other investigators who have relied heavily on the use of heat-killed MAP introduced either orally or parenterally. Investigators have avoided researching the possible use of live MAP vaccines because of the potential risks and liabilities associated with commercial use of a live MAP vaccine. In contrast to the present invention, previous approaches to MAP vaccine development have not utilized modified MAP strains having an attenuated virulence that could generate a sustained immunological response and long-term immunological memory without causing disease.

The Examiner has further rejected claims 1-6 under 35 U.S.C. 112 for not providing sufficient guidance into what is an "immunizing" dose or manner. The claims are herein amended to address this concern to state, "administering . . . a *controlled* dose and manner *to induce an immune response*." The Applicant asserts that the exact amount of inoculum and precise manner/schedule of its administration are not the crux of the invention. Although a typical dose for the inoculation of calves is in the neighborhood of 10^8 viable units [See *J. Comp. Path.*, Vol. 75, p. 281 (1965)], the exact optimum dose and manner of delivery could be readily determined by direct methods, once a MAP vaccine candidate is chosen. In fact, one skilled in the art would know that the USDA requires testing and determination of the appropriate dosing levels for each batch of vaccine produced. This means that the exact dosing levels will vary somewhat in practice according to the variations in virulence characteristics between different vaccine batches. Therefore, the exact dosing levels cannot be meaningfully determined at this time. Instead, the primary focus of the present invention is the development of a modified

MAP strain having an attenuated virulence created by either passaging the strain in culture or manipulating the strain through recombinant DNA techniques allowing for the effective oral administration of the modified MAP vaccine in generating the desired immunological response. An effective vaccine dose for a particular MAP vaccine strain is measured by approximating the amount of interferon-γ (γ-IFN) produced systemically in response to between about 10^6 to about 10^8 CFUs of MAP, a dosage which standardly establishes an infection in experimental animals. γ-IFN is one of the early products secreted by T cells to stimulate anti-mycobacterial macrophages during initial stages of cell-mediated immunity. [See *J. Vet. Diagn. Invest*, Vol. 8, p. 345 (1996)] However, the Applicant does not exclude the possible use of other methods that detect a specific immunological response to better enable the development of a MAP strain capable of eliciting the desired immunity to wild, disease-causing MAP.

In an alternative embodiment, a MAP organism could be killed by a non-protein denaturing technique, thereby preserving the ability of the killed MAP to adhere to the intestinal mucosa. Both of these approaches are distinguished from existing vaccines that use MAP strains killed by protein-denaturing heat inactivation and that generate only systemic immunity and limited local immunity when introduced parenterally. By contrast, a live, attenuated MAP strain (or a non-protein denatured, killed MAP) as proposed by the present invention would be introduced orally to the host animal and would generate a local and long-term immunological memory at the portal of infection (gastrointestinal mucosa) that would be capable of responding effectively against wild, disease-causing MAP strains.

In the previous Office Action, the Examiner stated that the *in vitro* attachment studies do not establish the successful *in vivo* administration of the vaccine. However, these attachment studies were merely intended to show that different MAP strains adhere differently to cells of the host intestinal tract. In particular, the high-passage MAP strain demonstrated an altered attachment affinity to intestinal explants. According to one embodiment of the present invention, these differences in attachment affinity between MAP strains (particularly high-passage MAP strains) would provide ***one means*** for the creation of a MAP vaccine with an attenuated virulence. In addition to changes in cell attachment affinity, passaging of MAP cells or direct manipulation of MAP via recombinant DNA techniques could also be used to modify ***other factors*** that affect the virulence of a potential MAP vaccine strain. Therefore, these *in vitro* attachment studies show that different MAP strains have variable infection characteristics and that these characteristics can be altered via multiple rounds of cell passaging and selection. In fact, the *Regg et. al.* research group in New Zealand has shown that MAP cells isolated directly from infected tissue were superior at establishing new infection than were passaged cultures. Strain F, disclosed in the current specification, is a high-passage strain having an altered attachment affinity to intestinal cells that could be used as a potential MAP vaccine candidate.

In an alternative embodiment of the present invention, a MAP strain could also be killed by a non-protein denaturing step prior to the administration of the MAP to the host organism. The advantage of this approach over prior art is that the protein component of the killed MAP is left largely intact by the non-denaturing conditions thereby allowing the non-protein denatured MAP to interact with the intestinal cells of the host animal,

which is necessary for the generation of the desired immunological response. The present specification describes the method for killing a MAP strain by non-protein denaturing means including the use of lethal irradiation (UV, γ-irradiation, etc.) as mentioned on line 16, page 6 of the originally filed application. This is contrary to existing methods for killing MAP strains by heat inactivation, which damages or destroys the conformational structures of MAP proteins required to interact with the host animal's intestinal cells, thereby compromising the ability of heat-killed MAP to generate a sufficient immunological response.

To overcome the Examiner's objection on 35 U.S.C. 112 grounds for confusion over the usage of the term "organism" in reference to both the inoculum strain and host, the "host organism" has been amended to "host animal." This should clearly distinguish the host animal from the inoculating MAP organism created by the described methods.

Lastly, the Examiner objects to Figures 4 and 6 in the Drawings section under 37 C.F.R. 1.83(a) for failing to show the individual attachment of strains B, E, and F to different explants of the gastrointestinal tract. However, as stated on lines 7-8 and 18-20 on page 16 of the original application, these strains did not show any statistical difference in their adherence to the explants and were, therefore, not provided individually. The data shown in these figures is accurately representative of the attachment affinity for each of the strains B, E, and F to the various gastrointestinal explants shown. In addition to the amendments described above, other amendments have been made to the specification to overcome the other objections by the Examiner. Accordingly, the Applicant asserts that the now amended claims 1-38 are in patentable condition.

CONCLUSION

In view of the foregoing, the amended claims 1-38, constituting the claims now pending in the application are submitted to be in patentably allowable condition and clearly stated in conformity with 35 U.S.C. 112 enabling requirements. If any issues remain outstanding, incident to the allowance of the application, Examiner Swartz is respectfully requested to contact the undersigned attorney at (919) 664-8222 or via email at jinang@trianglepatents.com to discuss the resolution of such issues, in order that prosecution of the application may be concluded favorably to the applicant, consistent with the applicant's making of a substantial advance in the art and particularly pointing out and distinctly claiming the subject matter that the applicant regards as the invention. This Office Action response is submitted via fax to the official group fax number 703-872-9306 on August 23, 2004.

Respectfully submitted,

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